European Journal of Medicinal Chemistry 46 (2011) 3942–3952

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Combination of pharmacophore model development and binding mode analyses: Identification of ligand features essential for IκB kinase-beta (IKKβ) inhibitors and virtual screening based on it

Hao-Peng Sun [a,b,c], Jia Zhu [a,b,c], Fei-Hong Chen [a,b,c], Sheng-Lie Zhang [a,b,c], Yuan Zhang [a,b], Qi-Dong You [a,b,c,*]

[a] Key Laboratory of Carcinogenesis and Intervention of Jiangsu Province, China Pharmaceutical University, Nanjing 210009, China
[b] Department of Medicinal Chemistry, China Pharmaceutical University, Nanjing 210009, China
[c] State Key Laboratory of Natural Medicines, China Pharmaceutical University, Nanjing 210009, China

ARTICLE INFO

Article history:
Received 9 December 2010
Received in revised form
2 April 2011
Accepted 26 May 2011
Available online 25 June 2011

Keywords:
IκB kinase β inhibitor
Pharmacophore
Homology model
Molecular docking
Virtual screening
Biological test

ABSTRACT

IκB kinase β (IKKβ) is an important anti-cancer target that plays crucial role in activating the transcription factor NF-κB in response to various inflammatory stimuli. In order to discover novel IKKβ inhibitors, a 3D chemical-feature-based QSAR pharmacophore model was established. A homology model of IKKβ enzyme was also developed to study the binding mode of IKKβ and its inhibitors. The two models were consistent in predicting the binding conformation of IKKβ inhibitor. Based on the virtual screening using the pharmacophore model, 16 compounds from SPECS database were selected after multiple filtrations for biological test. Two compounds with IC_{50} values lower than 10 μM were discovered.

1. Introduction

NF-κB plays a key role in regulating the transcription of many pro-inflammatory genes [1,2]. In the inactive form NF-κB resides in the cytoplam and associates with the IκB inhibitory proteins, which will be degraded in response to specific external stimuli [3–5]. The degradation of IκB proteins results in the release of active form of NF-κB into the nucleus, and sequentially induces the expression of more than 150 genes encoding cytokines, chemokines, cell adhesion proteins and proteases [6]. IκB kinase (IKK), a high molecular weight protein complex, is responsible for the phosphorylation of the IκB protein. The complex consists of two catalytic subunits (i.e. IKKα and IKKβ), one regulatory subunit called IKKγ or NEMO, NIK and other component proteins [7–9]. Although IKKα and IKKβ are highly homologous and contain similar structural domains, previous studies have shown that the IKKβ subunit plays a major role in NF-κB activation induced by pro-inflammatory stimuli such as TNF-α, IL1-β, IL-6, IL-17, and IL-23 [10,11]. Therefore, IKKβ is a potential therapeutic target for inflammation, cancer and other diseases. So far, several chemical families of synthetic IKKβ inhibitors have been identified (e.g. thiophenecarboxamides, pyridines, pyrimidines, indoles, quinazolines, benzimidazoles, and carboline derivatives [12]). These inhibitors provide a solid basis for elucidation of the quantitative structure–activity relationship (QSAR) and further identification of new molecules targeting IKKβ.

Computer-assisted technique has emerged as an important tool for drug design as it can provide target–ligand interaction information from both ligand-based and structure-based method. A large number of successful applications in medicinal chemistry have demonstrated the importance of these methods in drug design and research [13–18]. With the aim of gaining further insight into the structural requirements of IKKβ inhibitors and their interactions with the target enzyme, we developed a novel three-dimensional quantitative structure–activity relationship (3D-QSAR) pharmacophore model. This model highlighted important binding features of IKKβ ligands. To clarify the binding modes of inhibitors with IKKβ, a homology model was established for further docking study, and the conformations generated by the 2 models showed good consistency. The type and spatial location of chemical features encoded in the pharmacophore were consistent with the

* Corresponding author. Department of Medicinal Chemistry, China Pharmaceutical University, Nanjing 210009, China. Tel./fax: +86 025 83271351.
E-mail address: youqidong@gmail.com (Q.-D. You).

0223-5234/$ – see front matter © 2011 Elsevier Masson SAS. All rights reserved.
doi:10.1016/j.ejmech.2011.05.066

enzyme inhibitor interaction patterns identified by molecular docking. The 2 models generated from both ligand-based and structure-based method, respectively, provided deeply insight into the requirements of IKKβ inhibitors. Based on the virtual screening with the pharmacophore model, 16 compounds from SPECS database were selected after multiple filtrations for biological test. Most of them exhibited moderate inhibitory activity against IKKβ. Two compounds with IC_{50} values lower than 10 μM were discovered. Our study suggests that the pharmacophore model can provide guidance for the rational design to discover novel IKKβ inhibitors.

2. Materials and methods

2.1. General methodology

All compounds were optimized by Discovery Studio 2.5.5 software package (Accelrys Inc., San Diego, CA). Pharmacophore models were generated by using HypoGen module within Discovery Studio 2.5.5 on Dawning 560I workstation. The homology model of IKKβ was established by using MODELER inbuilt in Discovery Studio. Molecular docking was carried out by GOLD 3.01 program.

2.2. Training set and test set selection

To generate a rational pharmacophore model, the selection of the training set molecules must follow some rules: a) the set must be widely populated (at least 16 items) with structurally diverse representatives covering an activity range of at least four orders of magnitude; b) the most active compounds were inevitably included in the training set; c) the homogeneous procedures were applied to obtain all biologically relevant data. The data sets used in the present study were taken from several literatures [19–25]. 31 compounds (No. 1–31 in Chart 1), with IC_{50} ranging from 4 to 50,000 nM, were used as training set to generate HypoGen hypotheses. Another 29 IKKβ inhibitors with diverse activities and structures were selected as a test set to validate the pharmacophore model (No. 32–60 in Chart 2). The biological data (represented as IC_{50} values) of these inhibitors were determined under a similar experimental condition by using a radioactive P-labeled ATP kinase assay method. All molecules were built by Discovery Studio 2.5.5 software package. Multiple conformations of each compound were generated by using Diverse Conformation Generation protocol with an energy threshold of 20 kcal/mol and a maximum of 255 conformers.

2.3. Generation of pharmacophore hypotheses with 3D-QSAR pharmacophore generation (HypoGen)

The protocol HypoGen implemented in the Discovery Studio package was used to generate automated 3D-QSAR pharmacophore models. Based on the chemical features of compounds in the training set, the following chemical functions were selected in the feature dictionary: hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), hydrophobic (HY), and hydrophobic aromatic (HYAr) groups. The uncertain factor for each compound represents the ratio range of uncertainty in the activity value based on the expected statistical straggling of biological data collection. In this study, the default uncertainty value of 3 was used. Pharmacophores were then generated by using 3D-QSAR Pharmacophore Generation protocol, and the top 10 scoring hypotheses were exported.

The quality of 3D-QSAR pharmacophore models can be best described in terms of total cost, null cost, fixed cost and other statistical parameters. Total cost sums over error cost, weight cost and configuration cost. Null cost supposes that there is no relationship between the biological data and the pharmacophore model and that the experimental activities are normally distributed around their average value. While fixed cost represents a simple model that fits all data perfectly. For a reliable pharmacophore model, the total cost should be close to the fixed cost, and there should be a significant difference between null and total cost. Further, a value of 40–60 bits for the unit of cost difference implies a 75–90% probability of the correlation between experimental and predicted activities [26].

2.4. Homology modeling

The sequence of IKKβ protein catalytic domain was obtained from Swiss-Prot protein database (ID: O14920) [27]. The sequence similarity of IKKβ catalytic domain against protein databank sequences was analyzed by the NCBI BLAST server [28]. Four potential protein kinase catalytic domains were identified as homologs from PDB [29–31]. The automated sequence alignment and analysis of template and target was carried out by Align Structures protocol in Discovery Studio 2.5.5. MODELER inbuilt in Discovery studio package was used to model an IKKβ protein sequence. Four different kinase template sequences (PDB ID: 2GNF, 2HAK, 2J90, 2JC6) were aligned with IKKβ sequence and this alignment was supplied along with the 3D coordinates of templates as an input to the program. Modeler implemented comparative protein structure modeling by satisfaction of spatial restraints [32,33]. To assess the quality of the homology models, Ramachandran plot analysis was done by Discovery Studio 2.5.5.

2.5. Molecular docking

The binding interactions of IKKβ and its inhibitors were analyzed by the GOLD 3.01 program [34]. It employs genetic algorithm in which the information about the ligand conformation and hydrogen bonding is encoded in chromosome. GOLD considers complete ligand flexibility and partial protein flexibility and the energy functions are partly based on conformational and non-bonded interactions. Several types of scoring functions such as GoldScore, ChemScore and User-defined score are available. In this study, the following default genetic algorithm parameters were used: 100 population size, 1.1 for selection, 5 number of islands, 100,000 number of genetic operations and 2 for the niche size. Residues Leu21, Thr23, Gly24, Glu97, Cys99, Asp103, Lys147, Glu149, Asn150, Glu172 and 6.5 Å surrounding residues were defined as the active site, which completely covered the ATP binding pocket of IKKβ [35].

2.6. Database searching

Virtual screening of chemical databases facilitates discovering novel leads with the potential for further development. Compared to any other *de novo* design methods, database searching methodology provides the advantage that the retrieved compounds can be obtained easily for further biological testing [36]. In this study, we performed all database searching by using the Best/Flexible search option. The molecules fit all the features of the pharmacophore model were retained as hit. Geometric fit values were calculated for hit compounds based on how well the chemical substructures mapped on to the pharmacophoric feature location constraints and their deviation distances from the feature centers. High fit value indicated good match, and was used as the criteria for selecting compounds for further validation. Hit compounds with the fit values over 7.5 were analyzed for their drug-likeness properties by using Lipinski rule of 5 and ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) filters in Discovery